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Protective effects of luteolin on injury induced inflammation through reduction of tissue uric acid and pro-inflammatory cytokines in rats

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ABSTRACT

Background and aim: Luteolin belongs to flavone group of flavonoids, present in many plants with potent antioxidant, anti-inflammatory and anti-proliferative effects. The objective of present study was to investigate protective effect of luteolin on injury induced inflammation via Monosodium urate (MSU) crystals induced and Acetaminophen (AMP) induced liver injury in rats.

Experimental procedure: Protective effect of luteolin was observed by measurement of rat paw edema, lysosomal enzymes, antioxidants status and cytokine level. Measurement of uric acid level and neutrophil infiltration were done in AMP induced liver injury in rats. Luteolin was tested at 30 and 50 mg/kg doses and compare with colchicine.

Results and conclusion: Luteolin significantly decreases paw edema in dose dependent manner compare to control group in MSU crystal-induced rats. Luteolin (50 mg/kg) was showed significant decrease in serum level of oxidative and lysosomal enzymes, proinflammatory cytokines i.e. tumor necrosis factor (TNF)-α (39.28 ± 3.17), interleukin (IL)-1β (12.07 ± 1.24), and IL-6 (24.72 ± 2.52) in MSU crystal-induced rats. In AMP induced liver injury, tissue uric acid level and myeloperoxidase were decreased significantly after treatment with luteolin as well as N-acetylcysteine. Serum level of liver enzymes was significantly reduced after treatment with luteolin. Histological observation of ankle joints and liver was support to protective effect of luteolin at both doses. In conclusion, luteolin showed anti-inflammatory effect through restoration of cytokine level, lysosomal enzymes level and antioxidants status. The reduction of liver tissue uric acid content may be one of the mechanisms for protective effect of luteolin. It can contribute to reduce injury induced inflammation.

1. Introduction

In the innate immunity, the role of immune responses to the initiation of any injury inflammation, and infection has prominently increased. In comparison to the acquired immune system, innate responses notch the instant and early phases of host defense against foreign microbes as well as to injury, originating the inflammatory reaction. These developments focus our reflection to be linked between cells of the innate immune system and the products of tissue damage and cell death.[1,2] In an *in vivo* study, as a result of necrotic cell death it produces an acute inflammatory response that resulting further tissue damage and can cause disease condition. This inflammatory response induced by releasing pro-inflammatory intracellular components, such as uric acid.[3]

Gout arthritis is an inflammatory joint disorder which occurs due to increasing amount of uric acid deposited as monosodium urate crystals in the joints leading to an intense inflammatory process and pain. This deposition of uric acid occurs in the body due to poor metabolism of uric acid which leads to the formation of monosodium urate (MSU) crystals.[4] Diabetes, hypertension, obesity, cancer and hyperlipidaemia are the risk factors of arthritis. MSU crystals interact with various immune cells such as macrophages, neutrophils and synovial cells that induces the secretion of various inflammatory mediators such as tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1), IL-6, IL-8, and oxygen-derived free radicals, resulting in tissue damage. These cytokines are

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responsible to induce acute inflammation in gouty arthritic patients.[5] Currently nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids are frequently used for the management of pain and inflammation in acute gout. Indomethacin and naproxen are associated with many adverse reactions such as gastrointestinal toxicity, renal toxicity, or gastric and intestinal problems that limit their clinical uses.[6] Therefore, an effective and safe drug is required to explore against injury and chemical induced inflammatory disorders.

Medicinal plants can be a promising substitute for numerous diseases.[7–16] Luteolin, belongs to a group of naturally occurring flavonoids that are present in large proportion of many plants. It produces many medicinal properties such as antioxidant,[17] wound healing,[18] anti-inflammatory,[19] anti-bacterial, anti-diabetic[20] and anti-proliferative actions.[21] Luteolin belongs to the flavone group of flavonoids and has C6-C3-C6 structure. The hydroxyl moieties and 2–3 double bonds are important structure features in luteolin that are associated with its biochemical and biological activities.[22] Luteolin is often glycosylated in plants and the glycoside is hydrolyzed to free luteolin during absorption.[23] Various reports indicate that low concentrations of free luteolin can be achieved in plasma after oral ingestion of this flavonoid and most of the part converts into glucuronide and sulfateconjugates. To produce optimum biological effect, bioavailability of luteolin should sufficiently high and its metabolism sufficiently low.[24] In various *in vivo* studies related to luteolin, many researchers used intraperitoneal route due to greater bioavailability than the oral route. Previous reports showed that luteolin inhibits the lipid peroxidation and restores the antioxidant enzymes level during 1, 2-dimethyl hydroxide-induced colon cancer in rats.[25–27] Orally administration of luteolin significantly reduced the lipid peroxidation (LPO) and OH^- concentration in plasma and colonic mucosa as well as increases the antioxidant enzymes which might be due to the strong antioxidant property of luteolin.[17] In a report, luteolin has involved as a potent anti-inflammatory agent by modulating the inflammatory mediators.[28] But in chemically induced model luteolin decreased the expression of inducible nitric oxide synthase (iNOS) in rats,[29] and the mechanism is unknown. The objective of present study was to investigate protective effect of luteolin on injury induced inflammation in ankle joints and liver of rats.

2. Materials and methods

2.1. Chemicals and reagents

Myeloperoxidase (MPO) and N-acetyl-b, d-glucaminidase Kit from Krishgen BioSystems, Mumbai, India. Uric Acid Assay Kit purchased from BioAssay Systems, USA. Antioxidant Kit, ELISA Kit (TNF-α, IL-1β and IL-6 assay) were purchased from BIO Innovations, Mumbai, India. Acetaminophen, acid phosphatase, catalase, disodium phenyl phosphate, N-acetylcysteine, N-acetyl glucosaminidase, carboxy methyl cellulose (CMC), uric acid, luteolin, colchicine, 4-nitrophenyl-N-acetyl galactopyranoside, 4-nitrophenyl-N-acetyl glucosaminide, β-galactosidase, β-glucuronidase, p-nitrophenol, hexadecyl trimethyl ammonium bromide were purchased Sigma Aldrich Chemicals Pvt Ltd, Mumbai, India. All other common reagents and chemicals were used of the analytical grade and commercially available.

2.2. Animals

Wistar albino rats, (180–200 g) of either sex were selected for *in vivo* study. They were acclimatized for a week in a light and temperature-controlled room with a 12 h dark-light cycle. Animals were fed with commercial pelleted feed from Hindustan Lever Ltd. (Mumbai, India) and water was freely available. All animal experimental protocol approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, and Institutional Ethical Animal Committee, Chopda, Maharashtra India (Reg. No. NIB/IACE/09-10/86).

2.3. Synthesis of monosodium urate crystals

Accurate quantity of 4 g uric acid was dissolved in 800 mL of water with heating and pH adjusted to 8.9 by the addition of NaOH (9 mL/0.5 N), at 60 °C. The product was cooled up to overnight, washed and dried. After some time needle-like crystals were recovered and suspended in sterile saline (20 mg/mL). Before administration, MSU crystals were checked for bacterial endotoxin contamination using a commercial test kit (limulus amebocyte lysate, Pyrogent-5000, Biowhittaker Inc., USA).[30]

2.4. MSU crystal-induced inflammation in rats

Wistar albino rats were divided into five groups each containing of six animals. Group I, designated control group, received vehicle (0.5% carboxy methyl cellulose in phosphate buffered saline) only; Group II, was received endotoxin-free MSU crystal (4 mg/rat) suspension into the right foot pad by intradermal injection to induce inflammation.[31] Group III comprised of MSU crystal-induced rats were treated with luteolin (30 mg/kg body weight, i.p.). Group IV comprised MSU crystal-induced rats were treated with luteolin (50 mg/kg body weight, i.p.). Group IV comprised of MSU crystal-induced rats were treated with colchicine (1 mg/kg body weight, i.p.). Luteolin and colchicine were suspended in 0.5% carboxy methyl cellulose (CMC) in phosphate buffer saline (PBS) and were administered i.p., 1 h before the MSU crystal injection (single dose) and then once daily up to 3 days.

2.4.1. Assessment of paw thickness

The inflammation of all MSU crystals injected rats were measured by determining the paw thickness with a vernier scale at different intervals (0, 4, 24, 48 and 72 h) for 3 days.[31] After 72 h the animals were killed by cervical decapitation and blood samples were collected for serum separation and used to estimation of biochemical parameters like cytokines level, lysosomal enzymes, lipid peroxidation and antioxidants level.

2.4.2. Cytokines determination

Blood samples were collected from anesthetized rats of MSU crystal injected different groups by cardiac puncture and serum was separated by allowing the blood to clot at room temperature for 1 h, and then centrifuged at 2500 g for 10 min. The levels of TNF-α, IL-1β and IL-6 were measured by enzyme-linked immunosorbent assay (ELISA) kit in serum samples.[32]

2.4.3. Estimation of lysosomal enzymes

The serum obtained from blood samples of MSU crystal induced rats was also assessed for lysosomal enzyme activity. The activity of β-galactosidase was assayed by using 4-nitrophenyl-N-acetyl galactopyranoside as the substrate and activity was expressed as μmoles of p-nitrophenol liberated/h/mg protein.[33] N-Acetyl glucosaminidase activity was analyzed by using 4-nitrophenyl-N-acetyl glucosaminide as the substrate, and activity was expressed as μmoles of p-nitrophenol formed/h/mg of protein.[34] Acid phosphatase was assayed by using disodium phenyl phosphate and activity was expressed as μmoles of phenol liberated/min/mg protein.[35]

2.4.4. Estimation of lipid peroxidation and anti-oxidant status

Serum lipid peroxidation was analyzed by using a colouring agent, thiobarbituric acid. In this method, Malonaldehyde (MDA) used as an index of lipid peroxidation, produced during lipid peroxidation.[36] MDA reacts with thiobarbituric acid to produce a colouring product, which absorbs at 532 nm. Superoxide dismutase (SOD) activity in serum was assayed based on the inhibition of epinephrine autoxidation by the enzyme.[37] Serum catalase (CAT) activity was estimated by the method of Sinha (1972).[38] The activity of catalase was expressed as μg of H_2O_2 consumed/minute/mg protein, glutathione peroxidase estimated by the method of Rotruk et al. (1973).[39] Glutathione peroxidase was expressed as μg of glutathione utilized/minute/mg protein.

2.4.5. Histopathological study

The dissected rat ankles from different groups were washed with phosphate buffer and stored in 10% formalin. All samples were washed and dehydrated using isopropanol followed by xylene solution. The tissue samples were embedded in paraffin wax and sectioned (5 μm thickness) were subsequently stained with haematoxylin and eosin. All sections were examined under light microscope for histological examination in terms of joint space, inflammation and infiltration of inflammatory cells at the joints.

2.5. Acetaminophen-induced liver injury

Acetaminophen (AMP) solution (15 mg/mL in PBS) was made with heating at 55 °C. Rats were divided into 5 groups, each containing six rats. Group I is a normal control group and group II is a negative control group (acetaminophen hepatotoxicity control). Group III and IV were received acetaminophen induced hepatotoxicity rats with luteolin at 30 and 50 mg/kg respectively, daily up to 7 succeeding days prior to acetaminophen dose. Group V received acetaminophen induced hepatotoxicity rats with N-acetylcysteine. N-acetylcysteine was given in a dose of 300 mg/kg/day,[40,41] luteolin was given at dose of 30 and 50 mg/kg/day.[42] After overnight fasting, the animals were administrated intraperitoneally acetaminophen (300 mg/kg) solution and after 4 h later, rats were allowed for food. After 24 h, blood samples were collected from retro-orbital plexus for estimation of Alanine aminotransferase (ALT), aspartate alkaline phosphatase (ALP) and aminotransferase (AST) enzymes level by using standard kits.[43,44] After 7 days of all treatment, rats were sacrificed by cervical dislocation to separate liver samples for histology. One part of liver tissues was used for estimation of uric acid level and measurement of neutrophil and macrophage infiltration.[45]

2.5.1. Tissue uric acid measurement

The collected livers were immediately homogenized at 4 °C in PBS, at pH 7.4 to produced 10% homogenate. The liver homogenate was centrifuged at 5100 g for 15 min, for serum separation. Homogenates were filter through ultra filters (Millipore) and the filtrates were used for determining optical density at 595 nm by using plate reader (BioRad). Uric acid level was measured by Uric Acid Assay Kit (BioAssay Systems) according to standard procedure.[46]

2.5.2. Determination of neutrophil and macrophage infiltration

Myeloperoxidase (MPO) and N-acetyl-b, D-glucaminidase (NAGase) activities were used as indexes for determination of neutrophil and macrophage accumulation, respectively to investigate the possible cellular infiltration.[47] The tissue homogenates made from liver in 80 mM sodium acetate buffer (pH 5.5) containing 0.5% hexadecyl trimethyl ammonium bromide and held at 4 °C. The supernatant was collected after centrifugation at 20,000 rpm for 20 min. MPO activity was analyzed by spectrophotometer (Shimadzu Analytical India Pvt. Ltd, Mumbai) at 630 nm and NAGase activity was analyzed at 405 nm.

2.5.3. Histopathology of liver

After anesthesia, animals were sacrificed and abdominal cavities were opened for collection of livers. A portion of separated livers was used for histopathological study. Liver tissues were dehydrated using isopropanol followed by xylene solution, fixed in 4% formalin and embedded in paraffin according to the standard method. Paraffin embedded tissues were cut 6 μm thick section and stained with haematoxylin and eosin (H&E) for photomicroscopic observation.

2.6. Statistical analysis

The data from individual groups were presented as the mean ± SD. Differences between groups were analyzed using analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparisons test and minimum criterion for statistical significance was set at $P < 0.01$ and $P < 0.001$ for all comparisons.

3. Results

3.1. MSU crystal-induced inflammation in rats

3.1.1. Effect of luteolin on MSU crystal-induced rats paw edema

The effect of luteolin on MSU crystal-induced inflammation was assessed by measurement of rats paw edema up to 3 days (Fig. 1). The MSU crystal-induced rats paw swelling reach maximum after one day compared to control group which received only vehicle. In contrast, luteolin (30 and 50 mg/kg b.wt) administration to MSU crystal induced rats significantly decreased the paw edema in dose dependent manner gradually similar to colchicine treatment. The maximal protective effect was observed after 24 h with 50 mg/kg luteolin.

3.1.2. Effect of luteolin on cytokine level

Acute gouty arthritis is characterized by the marked expression of some pro-inflammatory cytokines. Inhibitory effect of luteolin on the serum level of pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) were measured in MSU crystal induced rats by ELISA (Table 1). Results were confirmed that luteolin treatment group (50 mg/kg) was showed significant decrease in the serum level of proinflammatory cytokines i.e. TNF-α (39.28 ± 3.17), IL-1β (12.07 ± 1.24), and IL-6 (24.72 ± 2.52) in MSU crystal-induced rats when compared to negative control group. Luteolin at 30 mg/kg have lesser effect on serum cytokines level of MSU crystal-induced rats. Effect of luteolin was also comparable to the colchicine treated group.

3.1.3. Effect of luteolin on lysosomal enzymes

Effect of luteolin on the release of lysosomal enzymes (β-glucuronidase, β-galactosidase, N-Acetyl glucosaminidase and acid Phosphatase) was observed in serum for 24 h (Table 2). MSU crystals stimulate the release of lysosomal enzymes. This stimulatory effect decreases significantly by luteolin. In other words, luteolin (50 mg/kg i.p.) significantly decreases the lysosomal enzymes level as approximately similar to the colchicine.

3.1.4. Effect of luteolin on lipid peroxidation and anti-oxidant status

The effect of luteolin on oxidative stress was investigated in MSU crystal-induced rats. We examined serum lipid peroxidation and anti-oxidants level (SOD, Glutathione peroxidase and CAT) of control and treated MSU crystal-induced rats (Table 3). The level of LPO was increased and antioxidants level was found decreased in treatment group of MSU crystal induced rats when compared to

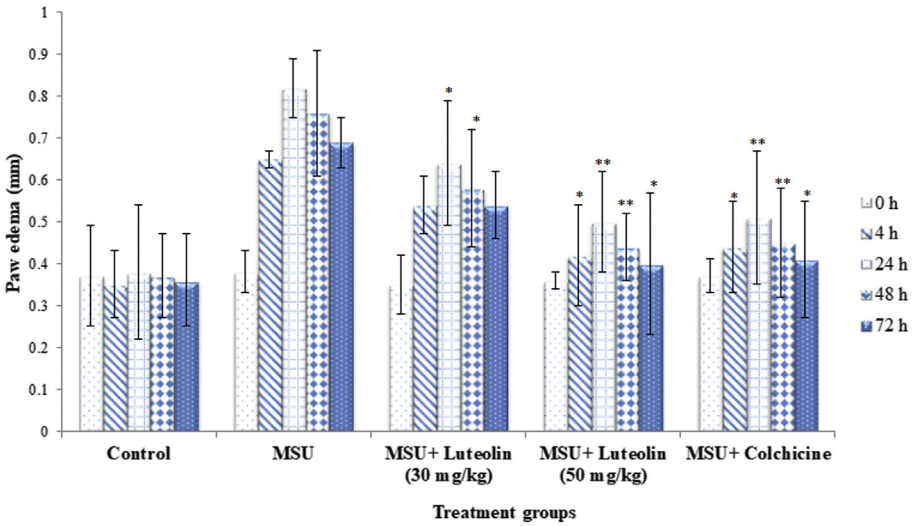

Fig. 1. Effect of Luteolin on monosodium urate (MSU) crystal-induced paw edema in rats. *P < 0.01, **P < 0.001, represent significant value compared with control group.

Table 1
Effect of Luteolin on cytokines level in monosodium urate crystal-induced in rats.

Groups	TNF-α(ng/mL)	IL-1β(ng/mL)	IL-6 (ng/mL)
Control	36.28 ± 2.04	11.57 ± 1.30	21.28 ± 1.85
MSU	68.24 ± 4.53	20.18 ± 2.19	39.35 ± 3.20
MSU + Luteolin (30 mg/kg)	54.37 ± 4.35	15.26 ± 1.16	29.37 ± 1.31*
MSU + Luteolin (50 mg/kg)	39.28 ± 3.17*	12.07 ± 1.24*	24.72 ± 2.52**
MSU + Colchicine	40.26 ± 2.51*	12.61 ± 1.07*	23.19 ± 1.37**

Values are presented as mean ± SD, *P < 0.01, **P < 0.001, represent significant value compared with control group. Abbreviation showed TNF-α, tumor necrosis factor- α; IL-1β, interleukin (IL)-1β.

control group. After treatment with 30 mg/kg and 50 mg/kg dose of luteolin treated MSU crystal-induced rats were showed significantly increased antioxidant status and reduced oxidative stress were found comparable to the colchicine effect.

3.1.5. Histopathological study

The results of histological assessment of joint section from rat's ankle were demonstrated that luteolin and colchicine could reduce the infiltration of inflammatory cells and erosive cartilage damage (Fig. 2). The control groups showed normal architecture of ankle joint of rat. MSU crystal induced rats observed reduced joint space with mild edema and marked articular erosion. Luteolin treated (50 mg/kg b.wt) rats showed increased joint space with minor articular erosion.

3.2. Effect of luteolin on acetaminophen-induced liver injury

The results of present study were indicated that luteolin could reduce the cell death induced liver inflammation in acetaminophen induced toxicity. After the administration of luteolin up to 72 h into the rats at therapeutic doses of 30 mg/kg and 50 mg/kg, we

Table 2
Effect of Luteolin on lysosomal enzymes activities in monosodium urate (MSU) crystal-induced rats.

Groups	β-glucuronidase (OD/g tissue protein)	β-galactosidase (OD/g tissue protein)	N-acetyl-β-Dglucasaminidase (OD/g tissue protein)	Acid Phosphatase (OD/g tissue protein)
Control	21.82 ± 1.84	17.48 ± 1.05	37.61 ± 2.41	4.28 ± 0.52
MSU	38.92 ± 1.28	28.67 ± 2.07	58.24 ± 3.28	7.43 ± 0.17
MSU + luteolin (30 mg/kg)	30.52 ± 2.41	23.85 ± 1.64	48.17 ± 3.67	5.84 ± 0.49
MSU + luteolin (50 mg/kg)	22.14 ± 1.82*	19.53 ± 1.27*	37.28 ± 2.16*	4.85 ± 0.27*
MSU + Colchicine	22.74 ± 1.76*	18.85 ± 1.09*	38.74 ± 2.81*	4.27 ± 0.61*

Values are presented as mean ± SD, *P < 0.01, represent significant value compared with control group.

Table 3
Effect of Luteolin on lipid peroxidation and antioxidants level in MSU crystal-induced rats.

Groups	Lipid peroxidation (nmol of MDA/mg of protein	SOD (Units/mg protein)	CAT (μg of H_2O_2 consumed/min/mg protein)	Glutathione peroxidase (μg of glutathione utilized/minute/mg/protein)
Control	1.57 ± 0.51	11.61 ± 1.34	23.85 ± 1.82	17.42 ± 1.02
MSU	5.36 ± 0.67	21.35 ± 1.29	41.35 ± 3.42	32.68 ± 2.18
MSU + Luteolin (30 mg/kg)	4.21 ± 0.18	18.49 ± 1.34	32.62 ± 2.61	28.42 ± 1.94
MSU + Luteolin (50 mg/kg)	2.58 ± 0.62*	13.54 ± 1.60*	25.83 ± 1.94*	19.63 ± 1.08*
MSU + Colchicine	2.79 ± 0.72*	12.94 ± 1.05*	24.67 ± 1.67*	17.87 ± 1.36*

Values are presented as mean ± SD, *P < 0.01, represent significant value compared with control group. Abbreviation showed SOD, Superoxide dismutase; CAT, Catalase; MDA, Malonaldehyde.

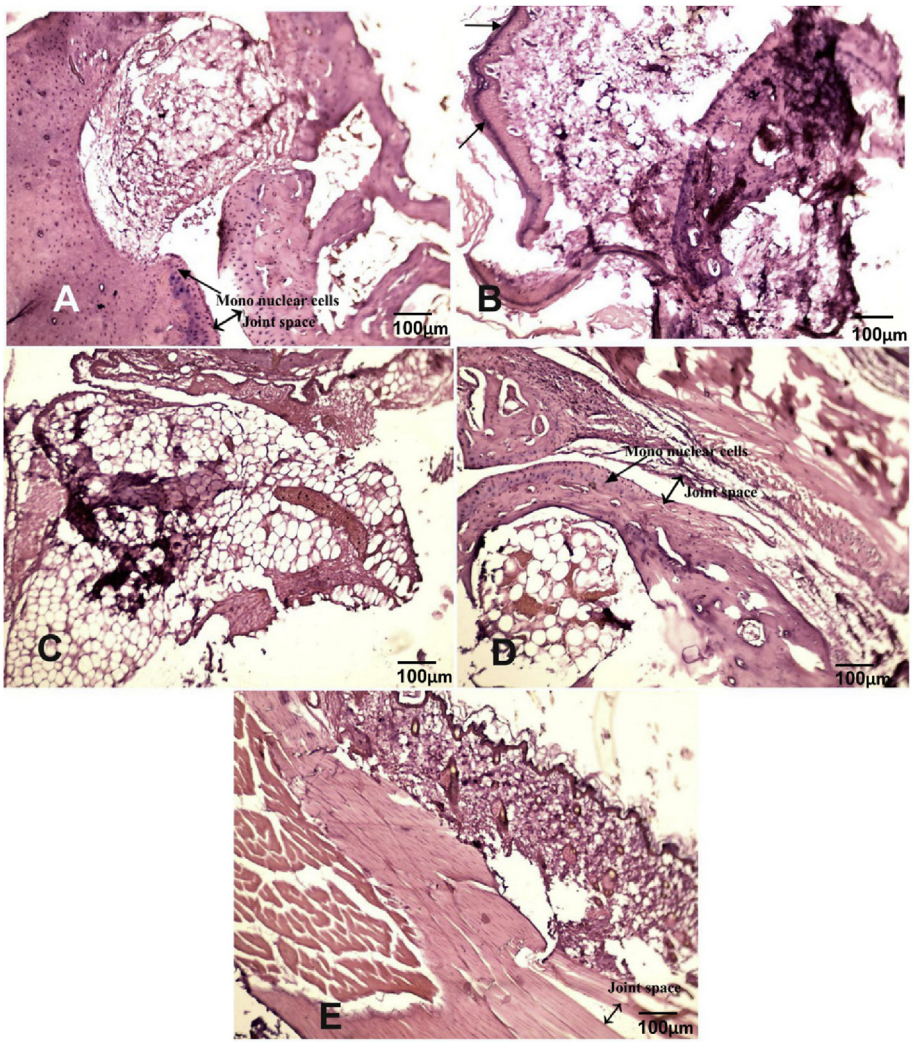

Fig. 2. Effect of luteolin and colchicine on histopathological changes in monosodium urate (MSU) crystal induced inflammation in ankle joints of rats: (A) Control group; (B) MSU; (C) MSU + Luteolin (30 mg/kg); (D) MSU + Luteolin (50 mg/kg); (E) MSU + Colchicine. Scale bars, 100 µm and Magnification, 100×.

measured uric acid content from all groups (Fig. 3). Luteolin reduces the uric acid level in liver, as expected at both doses. The higher dose 50 mg/kg of luteolin significantly reduced uric acid in liver tissue than lower dose.

Infiltration of neutrophils and macrophages were measured by the activity of marker enzymes MPO and NAGase, respectively in liver tissues (Table 4). According to our findings, MPO activity was significantly raised at 6 h after AMP administration. Among the various infiltrated cells, neutrophil is considered as a major source of pro-inflammatory mediators in AMP induced liver injury. Administration of AMP increases MPO activity in the negative control group of rats. Luteolin was found to declines MPO activity in dose dependent manner when compared with negative control group. Luteolin does not alter NAGase activity.

Liver enzymes (ALT, ALP and AST) in blood serum were estimated and showed a substantial increase in these enzymes level in serum after AMP induced liver injury (Fig. 3 and Table 5). By the treatment with luteolin 30 and 50 mg/kg doses, the enzymes level were significantly decreased as compare to negative control group of rats.

Histological observations of liver sections obtained from different animal groups are shown in Fig. 4. Normal control group of animals showed hepatic architecture with normal central vein. The cords of hepatocytes were separated by blood sinusoids lined with kupffer cells. The animal group treated with AMP showed dilated central vein, cellular centrilobular necrosis and massive inflammatory reactions. The liver sections from animals group treated with luteolin 30 mg/kg showed nearly normal architecture. The hepatocytes cells were observed normal with irregularly dilated and congested central vein. The group treated with luteolin at 50 mg/kg showed normal hepatocytes cells with dilated congested central vein and activated Kupffer cells. Liver sections obtained from animals treated with N-acetylcysteine showed nearly normal architecture and normal hepatocytes cells with minimal congested central vein and presence of activated Kupffer cells.

4. Discussion

Cell necrosis stimulates inflammation, and this phenomenon also connected with the pathogenesis of various diseases. Dead cells can release pro inflammatory molecules that are recognized by human immune system. Uric acid is an end product produced by the metabolism of endogenous and exogenous purine in humans.[1,48] Uric acid also stimulates different proinflammatory cytokines, such as interleukin-1β (IL-1β), IL-6 and tumor necrosis factor-α (TNF-α), in mononuclear cells. Uric acid content is

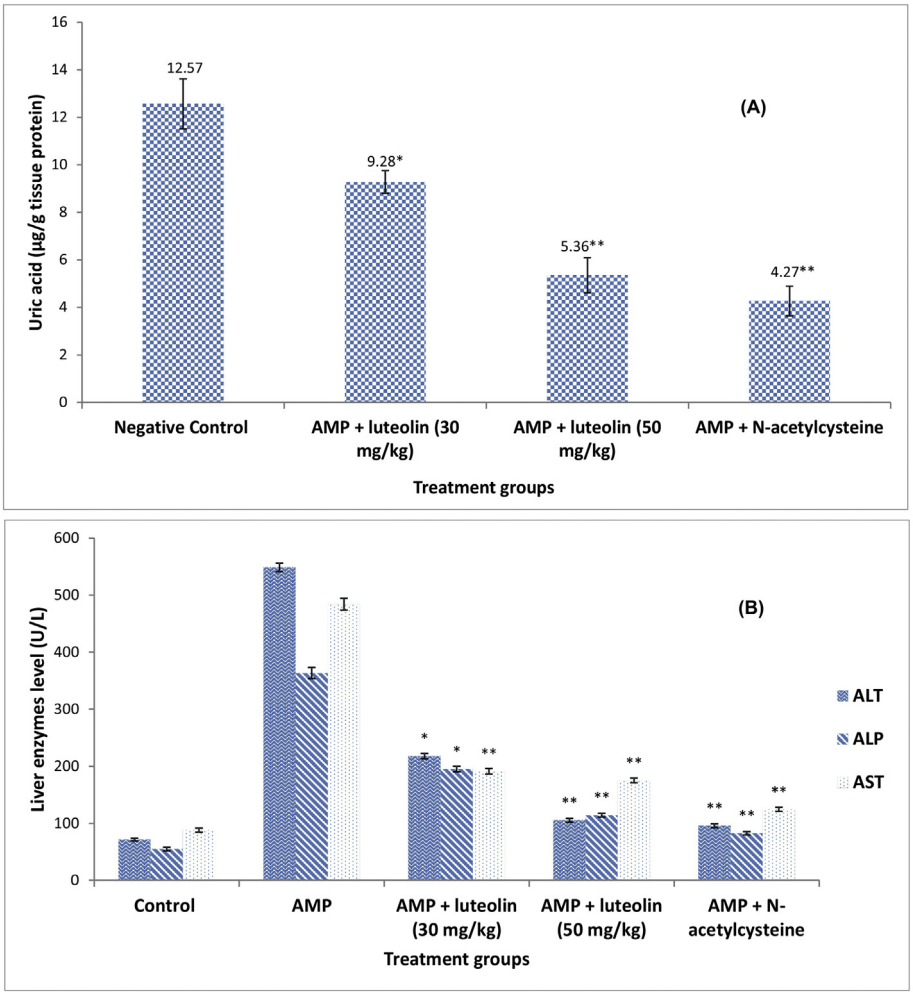

Fig. 3. Effect of luteolin on Acetaminophen (AMP) induced liver injury in rats: **(A)** Tissue uric acid level **(B)** Liver enzymes level. *P < 0.01, **P < 0.001, represent significant value compared with control group.

Table 4
Effect of luteolin on neutrophil and macrophage infiltration in acetaminophen (AMP) induce liver injury in rats.

Groups	MPO (OD/g tissue protein)			NAGase (OD/g tissue protein)		
	10 min	60 min	360 min	10 min	60 min	360 min
Control	3.52 ± 0.85	4.46 ± 0.75	5.17 ± 0.82	27.52 ± 1.75	28.61 ± 2.07	28.17 ± 2.10
AMP	3.81 ± 0.75	6.72 ± 0.52	9.53 ± 0.37	28.71 ± 2.63	29.34 ± 1.94	27.80 ± 1.85
AMP + luteolin (30 mg/kg)	3.62 ± 0.61	5.91 ± 0.37	8.43 ± 0.17	27.89 ± 1.94	28.01 ± 2.10	28.67 ± 2.09
AMP + luteolin (50 mg/kg)	3.49 ± 0.58	4.28 ± 0.49*	5.46 ± 0.68*	27.94 ± 1.82	28.37 ± 2.07	27.04 ± 1.83
AMP + N-acetylcysteine	3.73 ± 0.94	4.17 ± 0.61*	5.30 ± 0.83*	27.16 ± 1.38	28.40 ± 1.98	28.31 ± 1.57

Values are presented as mean of optical density (OD) ± SD, *P < 0.01, represent significant value compared with control group. Abbreviation showed MDA, Malonaldehyde; NAGase, N-acetyl-b, D-glucaminidase.

Table 5
Effect of luteolin on serum level of liver enzymes during acetaminophen (AMP) induce liver injury in rats.

Groups	Liver enzymes (U/L) level		
	ALT	ALP	AST
Control	71.42 ± 2.31	54.86 ± 3.20	88.16 ± 3.65
AMP	548.58 ± 7.61	363.18 ± 9.76	483.94 ± 10.53
AMP + luteolin (30 mg/kg)	217.69 ± 4.62*	195.34 ± 4.82*	191.25 ± 4.81**
AMP + luteolin (50 mg/kg)	105.35 ± 3.29**	114.20 ± 3.27**	175.24 ± 4.25**
AMP + N-acetylcysteine	95.73 ± 3.47**	82.47 ± 3.04**	124.38 ± 3.64**

Values are presented as mean ± SD, *P < 0.01, **P < 0.001, represent significant value compared with negative control group. Abbreviation showed MDA, Malonaldehyde; NAGase, N-acetyl-b, D-glucaminidase.

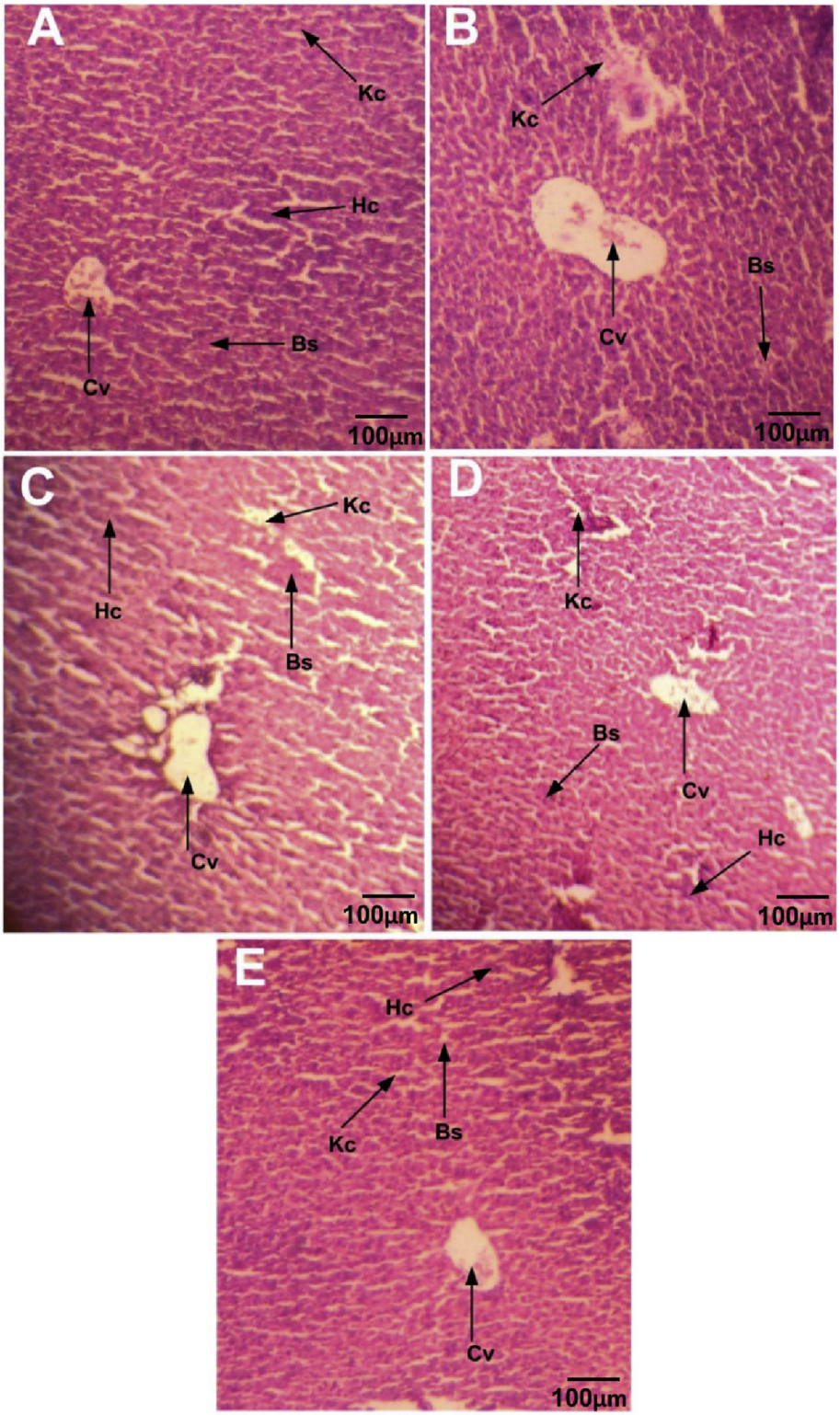

Fig. 4. Effect of luteolin and colchicine on histopathological changes in Acetaminophen (AMP) induced liver injury in rats: (A) Control group; (B) AMP; (C) AMP + Luteolin (30 mg/kg); (D) AMP + Luteolin (50 mg/kg); (E) AMP + N-acetylcysteine. (Abbreviation: Cv: Central vein; Kc: Kupffer cells; Hc: Hepatocytes cells; Bs: Blood sinusoids). Scale bars, 100 µm and Magnification, 100×.

associated with all these cytokines.[49]

It is believed that cells contain immune stimulatory molecules (known as damage-associated molecular patterns) that are released upon necrosis and not exposed in living cells.[50] Identifying molecular identity of these molecules and their role in inflammation is important to prevent or treat diseases initiated by cell death–induced inflammation. Hydrolytic enzymes and highly reactive molecule, such as oxygen radicals, leak from living and

dying leukocytes, and these molecules impose damage on cells present in the location. Researchers were reported that uric acid is released from dying cells as a proinflammatory molecule that contributes significantly to the cell death–induced inflammatory responses.[51] This would be consistent with the known proinflammatory properties of monosodium urate (MSU). It was previously reported that MSU and dead cells both stimulate sterile inflammation through the similar final pathway.[52,53] Therefore we have to select one of our study model i.e. MSU induced inflammation in rats. To study the role of uric acid in cell death–induced inflammation, a well-established acetaminophen induced liver injury model[51] was selected. Acetaminophen is metabolized in N-acetyl-p-benzoquinone imine in the liver that causes necrosis in the centrilobular regions of liver. The resulting sterile cell death stimulates some neutrophilic inflammatory response, which can be observed by measuring the content of myeloperoxidase (MPO) present in liver. We also determined the content of uric acid in the livers of controls and acetaminophen treated animals. Uric acid level was increased in the injured liver and this level was significantly reduced after treatment with luteolin.

Greater concentration of inflammatory mediators, such as TNF-α, IL-1β and IL-6 and cellular infiltrate rich in neutrophils are specific features of gouty arthritis. It has been reported that MSU crystals induces inflammation, by activating specific receptors via cytokines mediators released after activation of different cells.[5] Luteolin inhibited inflammatory response by reduced this elevated level of inflammatory mediators in MSU induced rat model. Literature showed that luteolin (20 and 50 mg/kg) significantly attenuated colon damage and significantly declined the expression of inflammatory mediators (iNOS, TNF-α and IL-6) and decreased MDA level. Luteolin significantly improved level of colonic SOD and CAT and the levels of nuclear factor-erythroid 2-related factor 2 (Nrf2).[42] These reports suggested that luteolin can be a potent anti-inflammatory agent. Therefore we aimed to explore the effect of luteolin on injury induced inflammatory disorders.

Increase in oxidative stress within inflamed joints is associated as mediator of inflammation and following induction of arthritis. It has been reported that during inflammation, different type of cells, e.g. macrophages and neutrophils have play major role to produce reactive oxygen species, that results to lipid peroxidation and imbalance to the anti-oxidant status.[54] So the free radicals are one of the critical determining factor that play important role in regulatory mechanism leading to the induction of pro-inflammatory mediators. Oxidative stress is one of the major causes for emerging acute and chronic disorders. Results were confirmed that after MSU administration, the levels of various tissue antioxidant enzymes like SOD, glutathione peroxidase and catalase were decreased significantly in rats. This declined in antioxidants enzyme level was due to increasing lipid peroxidation which also play a major role in severe arthritic conditions. Luteolin, is naturally occurring flavonoids, has been reported to possess strong antioxidative and anti-inflammatory activities.[55,56] In present study, luteolin treatment reduces oxidative stress by inhibition of lipid peroxidation therefore reduced the activity of SOD, CAT and glutathione peroxidase enzymes.

The MSU crystals induced inflammation model was selected, because the formation of MSU in the joint synovium initiates inflammatory response in joint and leukocyte infiltration, which is the main characteristic of gouty arthritis.[57] Among the various infiltrated cells, neutrophil is considered as a major source of pro-inflammatory mediators in MSU crystal-induced inflammatory response.[58,59]

MSU crystals stimulated to the macrophages that cause rupture of lysosomal membrane. It results the release of lysosomal enzymes (β-glucuronidase, β-galactosidase, N-Acetyl glucosaminidase and acid Phosphatase) and increase the level of these enzymes in blood. These enzymes are also responsible for inflammation, degradation of proteins, glycosaminoglycans and lipids. Therefore, decrease in the level of extracellular lysosomal enzymes could be favorable for control of arthritic inflammation.[31] In present study, luteolin significantly reduces the activities of lysosomal enzymes in MSU crystal-induced rats. Luteolin restore the levels of lysosomal enzymes close to normal level by stabilizing the lysosomal membrane.

In present investigation, it was confirmed that luteolin can decrease intracellular uric acid level and inhibit cell death-induced inflammation in acetaminophen induce liver injury in rats. Sometimes at high doses of acetaminophen causes hepatocyte necrosis.[55,60] This is the basis for induction of a toxic metabolite, N-acetyl-pbenzo-quinone imine, after metabolism of drug in the liver. Acetaminophen-induced hepatic necrosis stimulates inflammatory reactions and this progression is initiated by the release of uric acid from dead cells. These conditions can cause to liver dysfunction or failure.[46] The purpose of measurement of uric acid level in liver tissue is mainly due to the release of intracellular uric acid which has been associated in the developed inflammation after the organ injury. MPO is a hemoprotein that is abundantly expressed in neutrophils and present in monocytes and some macrophages. It is used as an indicator of neutrophil accumulation. It is already reported that MPO retains potent pro-inflammatory properties and may contribute to tissue injury.[61] Liver enzymes are good indicators for liver functioning and assessment of these enzymes in the serum is useful tool for hepatic damage. If the liver enzymes level increased, it may results liver damage.[62] After intraperitoneally administration of luteolin at 30 and 50 mg/kg doses, significantly reduced the enzymes level in blood that indicates the protective role of luteolin.

The most effective antidote used for acetaminophen detoxification is N-acetylcysteine (NAC). It is also a precursor for cellular glutathione synthesis and reported good known antioxidant.[48] We found that levels of uric acid significantly increased after acetaminophen-induced liver injury in rats. After treatment with luteolin, it was inhibited significantly injury-induced promotion in tissue uric acid level.

5. Conclusion

In summary, results of present study were confirmed that luteolin reduces tissue uric acid level in acetaminophen induced liver injury. Our results supported that uric acid as a proinflammatory molecule released from dying cells that contributes significantly to the cell death–induced inflammatory process. In addition, luteolin was able to significantly decreased neutrophil infiltration and pro-inflammatory cytokines production and also reduced oxidative stress in MSU crystal induced inflammation. Based on these findings, luteolin may be a potential preventive or therapeutic candidate for the treatment of arthritic inflammation and inflammatory mediated disorders.

Conflicts of interest

The author has declared no conflict of interest.

Compliance with ethics requirements

All animal experimentation was carried out according to CPCSEA guidelines recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India. All protocol was approved by Institutional Ethical Animal

Committee (Reg. No. NIB/IACE/09-10/86).

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